DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

Cindy Green
Regulatory Affairs Consultant
Northwest Regulatory Support
P.O. Box 1277
Maple Valley, WA 98038

MAR 31 1998

Re: P970037
AutoDELFIA™ hAFP Test Kit
Filed: August 26, 1997
Amended: September 29, October 9, and November 26, 1997; January 26 and March 27, 1998.

Dear Ms. Green:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the AutoDELFIA™ hAFP Test Kit. The AutoDELFIA™ hAFP Kit is intended for the quantitative determination of human alpha-fetoprotein (AFP) in maternal serum and amniotic fluid obtained between the 15th and 21st weeks of gestation. The assay is to be performed on the 1235 AutoDELFIA™ automatic immunoassay system, and is intended for use only in conjunction with other diagnostic tools such as ultrasound and amniography as an aid in the detection of Open Neural Tube Defects (ONTDs). We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

Expiration dating for this device has been established and approved at twelve (12) months.

CDRH will notify the public of its decision to approve your PMA by making available a summary of the safety and effectiveness data upon which the approval is based. The information can be found on the FDA CDRH Internet HomePage located at http://www.fda.gov/cdrh/pmapage.html. Written requests for this information can also be made to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857. The written request should include the PMA number or docket number. Within 30 days from the date that this information is placed on the Internet, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by the independent advisory committee, under section 515(g) of the Federal, Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Peter E. Maxim, Ph.D. at (301) 594-1293.

Sincerely yours,

Kimber C. Richter

Kimber C. Richter, M.D.
Deputy Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

Issued: 3-4-98

CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the addition of, but not the replacement of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant(s) involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

(1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).

(2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:

> (a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and
>
> (b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

(1) A mix-up of the device or its labeling with another article.

(2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and

(a) has not been addressed by the device's labeling or

(b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.

(3)Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984. This regulation was replaced by the reporting requirements of the Safe Medical Devices Act of 1990 which became effective July 31, 1996 and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to the FDA whenever they receive or otherwise become aware of information, from any source, that reasonably suggests that a device marketed by the manufacturer or importer:

(1)May have caused or contributed to a death or serious injury; or

(2)Has malfunctioned and such device or similar device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for a PMA, the manufacturer shall submit the appropriate reports required by the MDR Regulation within the time frames as identified in 21 CFR 803.10(c) using FDA Form 3500A, i.e., 30 days after becoming aware of a reportable death, serious injury, or malfunction as described in 21 CFR 803.50 and 21 CFR 803.52 and 5 days after becoming aware that a reportable MDR event requires remedial action to prevent an unreasonable risk of substantial harm to the public health. The manufacturer is responsible for submitting a baseline report on FDA Form 3417 for a device when the device model is first reported under 21 CFR 803.50. This baseline report is to include the PMA reference number. Any written report and its envelope is to be specifically identified, e.g., "Manufacturer Report," "5-Day Report," "Baseline Report," etc. Any written report is to be submitted to:

Food and Drug Administration
Center for Devices and Radiological Health
Medical Device Reporting
PO Box 3002
Rockville, Maryland 20847-3002

Copies of the MDR Regulation (FOD # 336&1336)and FDA publications entitled "An Overview of the Medical Device Reporting Regulation" (FOD # 509) and "Medical Device Reporting for Manufacturers" (FOD #987) are available on the CDRH WWW Home Page. They are also available through CDRH's Fact-On-Demand (F-O-D) at

800-899-0381. Written requests for information can be made by sending a facsimile to CDRH's Division of Small Manufacturers Assistance (DSMA) at 301-443-8818.

Summary of Safety and Effectiveness

I. General Information

Device generic name: Fluoro immunoassay for *in vitro* diagnostic quantitation of alpha fetoprotein in maternal serum and amniotic fluid.

Device trade name: AutoDELFIA™ hAFP Kit

Applicant's name and address: Wallac Oy
Mustionkatu 6
Turku, Finland
20750

U.S. Representative:
Cindy Green
Northwest Regulatory Support
P.O. Box 1277
Maple Valley, WA
98038

PMA number: P970037

Date of panel recommendation: Pursuant to section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Immunology Advisory Panel meeting because the information in the PMA substantially duplicates information previously reviewed by this panel.

Date of notice of approval to the applicant: March 31, 1998

II. Indications for Use

The AutoDELFIA™ hAFP kit is intended for the quantitative determination of human alpha-fetoprotein (AFP) in maternal serum and amniotic fluid obtained between the 15th and 21st weeks of gestation. The assay is to be performed on the 1235 AutoDELFIA™ automatic immunoassay system, and is intended for use only in conjunction with other diagnostic tools such as ultrasound and amniography as an aid in the detection of Open Neural Tube Defects (ONTDs).

Background

Human alpha-fetoprotein (hAFP) is a major protein component of embryonic serum expressed during fetal development. Human alpha-fetoprotein (hAFP) is first synthesized by the embryonic yolk sac cells and later by the fetal liver[1-2]. It is also found in the fetal cerebrospinal fluid[1-7]. It diffuses to the maternal blood by way of the amniotic membrane and reaches the amniotic fluid through fetal urine. The level of hAFP in amniotic fluid and maternal serum is elevated in connection with certain fetal malformations, especially open neural tube defects. Synthesis increases steadily during pregnancy to peak at a fetal plasma level of a few grams per liter at 10 to 13 weeks, declining to less than 100 µg/L by two years of age. The synthesis of AFP decreases in late fetal life as albumin (ALB) synthesis begins. ALB becomes the major component of adult human serum. Reappearance of AFP in adult serum is now recognized as an indicator of pathological conditions, including open neural tube defects in pregnancy[1,2], hepatocarinomas, teratocarcinomas, hereditary tyrosinemia, and ataxia telangiestasia[1-7]. Alpha-fetoprotein's biological function is unknown, although it may play a role in prevention of immune rejection of the fetus by the mother[19].

Concentrations of AFP are estimated as follows:

Source of AFP	AFP Level
Adult serum	50 ng/mL
Maternal serum	10-100 ng/mL
Amniotic fluid	10-30 µg/mL
Cord serum	10-200 µg/mL
Fetal blood	<1 mg/mL

The primary sequence of human AFP was determined initially through a cDNA clone of AFP mRNA and was shown to have 590 amino acids with a calculated molecular weight of 66,300 Daltons[8]. It has a carbohydrate content of 3-4%. The relative proportions of sialic acid, mannose and galactose residues may vary, causing electrophoretic microheterogeneity as well as heterogeneity with respect to lectin binding. The amino acid composition displays about 40% homology with human albumin, and they have similar physical and chemical

properties, but their immunological properties are clearly distinct from each other[9-11].

Since the mid-1980s, the use of maternal serum alpha-fetoprotein (MSAFP) in prenatal screening programs for anencephaly and spina bifida had become the standard of care for all pregnant women[12]. At the most commonly applied cut-off levels, MSAFP screening detects about 85% of open NTDs, 81% of fetuses with spina bifida, and 90% of those with anencephaly[13]. Screening for MSAFP will not detect skin-covered defects, also known as closed defects (i.e., most encephaloceles)[14].

There are a number of factors that may cause alterations in MSAFP levels. Compromises in AFP synthesis, the feto-amniotic fluid or placental barrier, or in the placenta itself, may cause alterations in the MSAFP. In fact, most elevations in MSAFP are the result of placental disease, cystic changes or simply large placenta size, all of which increase that organ's permeability. Other causes of elevated MSAFP include fetal ventral wall defects, multiple pregnancy, fetal death, vaginal bleeding, and hereditary Finnish nephrosis, a rare congenital renal disorder[20].

Relationships have been demonstrated[21] between unexplained MSAFP elevation and preterm deliveries, small for gestational age (SGA) and extremely SGA fetuses. In these cases, a high MSAFP is thought to be due to increased transplacental AFP transport into the maternal circulation, due to either leakage through a defective feto-maternal placental barrier or an increase in the placental villous surface area. A similar relationship in twin pregnancies was reported[22].

The measurement of MSAFP has been most specific, however, in the diagnosis of "open" neural tube defects (ONTDs). Failure of the neural tube to close results in large quantities of AFP entering the amniotic fluid through the cerebrospinal fluid, presumably because tissues like the choroid plexus have direct access to the exterior[23].

III. Device Description

The AutoDELFIA™ hAFP assay is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique in which two murine monoclonal antibodies are directed against two separate antigenic determinants on the AFP molecule.

Kit standards, serum or amniotic fluid samples are reacted simultaneously with immobilized monoclonal antibodies on the microtitration plate and europium-labeled (tracer) monoclonal antibodies which are added to the reaction well. If AFP antigen is present in the specimen, a "sandwich" of binding occurs between the antigen and both antibodies, since the antibodies react with different epitopes located on the antigen.

Following an incubation of the sample and tracer, the microtitration plate is washed and Enhancement Solution is added. Enhancement Solution dissociates the europium (Eu) ions from the labeled antibody and allows Eu to form highly fluorescent chelates with ingredients of the Enhancement Solution. The fluorescence from each specimen is proportional to the concentration of the hAFP in the specimen.

The assay may be run either with a full set of standards or with two calibrators in conjunction with an established reference curve. The specimen value is determined by comparison of the counts per second (cps) to concentration (U/mL) plotted on the standard curve.

Each laboratory is directed to establish their own reference ranges and medians for each gestational week. Each laboratory assays approximately 100 specimens for each gestation week from 15 to 21. A median is determined and multiples of the median are established to determine a positive result which requires additional follow up.

Results which are greater than the cut-off lead to a series of follow-up investigations. In the event the specimen is positive, a repeat serum sample is taken one week later. If the specimen repeat is high, a sonogram is recommended. Using the sonogram, the gestational age can be confirmed as

well as a condition of multiple pregnancy. If there is no evidence of error in gestational age or multiple pregnancy, an amniotic fluid assay for AFP is recommended.

CONTRAINDICATIONS:
There are no known contraindications for the AutoDELPHIA™ hAFP assay.

WARNINGS AND PRECAUTIONS:
Warnings and precautions for use of the device are stated in the attached product labeling. (See labeling)

IV. Alternative Practices and Procedures

Alternative practices and procedures used to aid in the diagnosis of ONTD include diagnostic ultrasound and amniocentesis. In addition, there are other immunological *in vitro* diagnostic devices for which there are approved PMAs for the quantitative determination of AFP in maternal serum and amniotic fluid specimens.

V. Marketing History

The manual DELFIA® hAFP kit was first introduced in Europe in 1985 and in 1993 the automated version, the AutoDELFIA™ hAFP kit, was launched. Neither version has been introduced in the U.S.

The product is currently sold in approximately two dozen different areas (i.e., countries or regions such as Eastern Europe). The primary markets for the kit are the following: United Kingdom, Eastern Europe, Germany, Italy, Australia, France, Africa, Brazil, and Finland.

VI. Adverse Effects of the Device on Health

As with any *in vitro* diagnostic device, a possible adverse effect may be either a false positive or false negative result.

In the event of an initial positive result, confirmatory testing is recommended in the package insert. In order for a false positive result to be reported (incorrect diagnosis of an ONTD), the confirmatory test would also have to report a positive result which would also be incorrect.

In the event of a false negative report, a fetal abnormality would not be detected because no further testing would be done and the pregnancy outcome could potentially be a birth with an ONTD condition.

VII. Summary of Studies

A. Summary of nonclinical studies

Characterization of the Antigen

The AFP antigen was obtained from human cord blood, a source rich in AFP, a single chain glycoprotein with a molecular weight of approximately 65,000 Dalton. Antigen was analyzed for performance as compared to secondary standards in the hAFP assay. The secondary standards are calibrated against a WHO International AFP Standard. The assay utilizes two antibodies [one coated in the wells of a microtitration plate and the other labeled with Europium in the tracer]. Each of the two antibodies recognize a different epitope on the AFP molecule.

The antigen is received as pooled, frozen, unpurified material and kept frozen until further processing. The material is tested for HIV and Hepatitis prior to receipt. The cord serum is filtered and diluted prior to testing to determine the AFP concentration. Once the concentration of the cord serum is determined, the material is further diluted to prepare the series of standards used in the hAFP test kit.

Characterization of the Antibodies

Immunogen for generation of the AF5/A2 monoclonal antibody was human alpha fetoprotein derived from amniotic fluid. Hybridomas were prepared by fusion of X63-Ag 8.653 murine myeloma cells with immune splenocytes of the immunized Balb/c mice. Hybridomas were cloned and assessed for contamination

[bacterial, fungal, viral, mycoplasma] and characterized with respect to growth properties [doubling times, ascites production, etc.].

Antibody AF5/A2 was purified by affinity chromatography and subjected to biochemical characterization [native PAGE, reducing SDS PAGE, isoelectric focusing] and immunological characterization [performance testing in the AutoDELFIA™ hAFP kit]. AF5 has been isotyped as IgG1.

Immunogen for generation of the HY 34/23 monoclonal antibody was hAFP purified from cord blood serum. Hybridomas were prepared by fusion of X63-Ag 8.653 murine myeloma cells with immune splenocytes of the immunized Balb/c mice. Hybridomas were cloned and assessed for contamination [bacterial, fungal, viral, mycoplasma] and characterized with respect to growth properties [doubling times, ascites production, etc.].

Antibody HY 34/23 was purified by affinity chromatography and subjected to biochemical characterization [native PAGE, reducing SDS PAGE, isoelectric focusing] and immunological characterization [performance testing in the AutoDELFIA™ hAFP kit]. HY 34/23 has been isotyped as IgG1.

Assay Performance Studies

Reproducibility Studies

1. Clinical sites

A panel of four serum samples was tested with hAFP at two sites: B.C. Children's Hospital (BCCH), Vancouver, British Columbia, Canada and Foundation for Blood Research (FBR), Scarborough, Maine. For each sample, two runs per day with two replicates of each sample were tested over a 20-day period with one kit lot of hAFP to assess intra/inter-assay, day to day and combined reproducibility.

Variance components methods were used to estimate the intra-assay (within run), and inter-assay (between run and day) reproducibility both separately for each site and pooled across the sites. Between-site reproducibility was further assessed in the latter analysis. The results are presented in Tables 1 and 2.

Table 1: Reproducibility by Site

		Within Run		Between Run		Between Day		Total	
Sample	Mean	Variance	CV*	Variance	CV	Variance	CV	Variance	CV
BCCH									
1	24.512	0.070	1.08%	0.086	1.20%	0.018	0.55%	0.173	1.70%
2	52.220	0.274	1.00%	0.840	1.75%	0.198	0.85%	1.293	2.18%
3	102.233	0.831	0.89%	1.630	1.25%	0.876	0.92%	3.282	1.77%
4	278.449	2.378	0.55%	13.915	1.34%	0.000	0.00%	15.903	1.43%
FBR									
1	24.726	0.060	0.99%	0.059	0.98%	0.059	0.98%	0.174	1.69%
2	52.330	0.363	1.15%	0.355	1.14%	0.131	0.69%	0.840	1.75%
3	102.934	0.578	0.74%	1.006	0.97%	0.610	0.76%	2.158	1.43%
4	274.820	16.314	1.47%	15.872	1.45%	3.769	0.71%	35.611	2.17%

*CV = (square root of the variance / mean) x 100

Table 2: Overall reproducibility across sites

Sample	Overall Mean	Within Run		Between Run		Between Day		Between Site		Total	
		Var.	CV*	Var.	CV	Var.	CV	Var.	CV	Var.	CV
1	24.619	0.065	1.03%	0.073	1.09%	0.038	0.80%	0.018	0.55%	0.184	1.74%
2	52.273	0.318	1.08%	0.597	1.48%	0.165	0.78%	0	0.00%	1.063	1.97%
3	102.583	0.704	0.82%	1.318	1.12%	0.743	0.84%	0.167	0.40%	2.826	1.64%
4	276.636	9.346	1.11%	14.893	1.40%	1.773	0.48%	5.997	0.89%	28.903	1.94%

Var. = Variance

*CV = (square root of the variance / mean) x 100

It can be seen that the within run, between run, between day, and total coefficients of variation (CVs) all were less than 2.2% for all 4 samples at both sites (Table 1). This situation remained true when results from both sites were analyzed together (Table 2). The between site CVs were less than 1%, and the total CVs across both sites were less than 2% for all 4 samples. Overall, the assay was reproducible with acceptable precision in the range tested.

2. In-House Reproducibility (Intra-assay, inter-assay and lot to lot variability)

Studies were performed at Wallac on six serum controls, three serum pools and three diluted amniotic fluid samples. Three kit lots were used on three AutoDELFIA™ systems for three days. One evaluation was performed using a full standard curve and the other was using a two-point calibration. The results are as follows.

Table 3: Variability using a full standard curve on each plate

Sample/control	Mean U/mL	Intra-assay CV [%]	Inter-assay CV [%]	Total CV [%]	Lot to Lot CV [%]
Lyphocheck 40032	88.19	0.94	2.28	2.46	1.21
Lyphocheck 40033	171.6	0.73	2.11	2.24	1.25
QA 1	2.30	1.18	2.21	2.51	1.15
QA2	62.27	0.84	1.92	2.09	0.67
QA3	440.1	1.06	2.06	2.31	0.67
QA4	21.9	0.97	2.03	2.25	0.19
Serum 1	21.86	0.98	1.67	1.93	0.23
Serum 2	129.2	0.92	1.95	2.16	0.88
Serum 3	382.1	1.09	2.21	2.47	1.33
AF 1	9258	1.35	1.21	1.81	0.22
AF2	8491	1.27	2.02	2.39	0.39
AF3	8979	1.48	1.67	2.23	0.69

Table 4: Variability using the lot specific reference curve and two calibrators (10 and 500 U/mL)

Sample/control	Mean U/mL	Intra-assay CV [%]	Inter-assay CV [%]	Total CV [%]	Lot to Lot CV [%]
Lyphocheck 40032	87.84	0.89	2.34	2.51	1.42
Lyphocheck 40033	170.8	0.73	2.18	2.30	1.12
QA 1	2.35	1.18	1.73	2.09	0.81
QA2	62.29	0.82	2.33	2.47	1.41
QA3	435.5	1.06	2.02	2.29	0.58
QA4	22.05	0.95	2.13	2.33	0.58
Serum 1	22.01	1.01	1.53	1.84	0.66
Serum 2	128.7	0.92	2.19	2.37	1.23
Serum 3	378.5	1.06	1.96	2.23	0.67
AF 1	9277	1.36	1.75	2.21	1.41
AF2	8477	1.25	2.46	2.76	1.64
AF3	8933	1.44	1.90	2.39	0.84